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INHIBITORS OF PROSTAGLANDIN BIOSYNTHESIS FROM DALBERGIA ODORIFERA

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From the heartwood of Dalbergia odorifera T. Chen, a Chinese medicinal drug (Japanese name "Koshinko") used for the stagnation of disordered blood, five new phenolic compounds, isomucronustyrene (**2**) and hydroxyobtustyrene (**3**) (cinnamylphenol), (±)-isoduartin (**6**) (isoflavan), odoriflavene (**7**) (isoflav-3-ene) and (-)-odoricarpan (**15**) (pterocarpan) were isolated and their structures were elucidated on the basis of chemical and spectroscopic methods. Of the fifteen compounds isolated, the cinnamylphenols, isoflavans, isoflav-3-ene and a benzoic acid derivative inhibited prostaglandin biosynthesis more than the pterocarpan and isoflavone compounds.

KEYWORDS——Dalbergia odorifera; Leguminosae; stagnant blood; cinnamylphenol; isoflavonoid; isoflavan; isoflav-3-ene; pterocarpan; prostaglandin biosynthesis inhibitor; platelet aggregation inhibitor

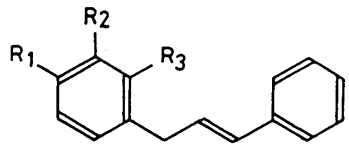
In the course of our screening works to find biololgically active principles contained in medical drugs used in traditional medicines, the hot aqueous extract of the heartwood of Dalbergia odorifera T. Chen (Japanese name "Koshinko"), which has been used as a Chinese medical drug to treat the stagnation of disordered blood, was found to inhibit prostaglandin (PG) biosynthesis (98% inhibition at 0.75 mg/ml). It has been recognized that the balance between thromboxane A_2 and PG I_2 has a very important role in the regulation of blood flow. The isolation of inhibitors of PG biosynthesis from this medical plant provides a substantial support for its usage.

The heartwood of D. odorifera purchased in the Taipei market was extracted with methanol and the methanol extract was fractionated into n-hexane-, chloroform- and water-soluble fractions by the usual procedure. A benzene-soluble fraction obtained from the chloroform-soluble fraction had the most potent inhibitory effect to PG biosynthesis.[1] Separation and purification by repeated column chromatography, along with testing the inhibitory effects, gave obtustyrene (**1**), isomucronustyrene (**2**), hydroxyobtustyrene (**3**), (±)-mucronulatol (**4**), (±)-vestitol (**5**), (±)-isoduartin (**6**), odoriflavene (**7**) and methyl 2-hydroxy-3,4-dimethoxybenzoate (**8**), as active principles. In the course of separation of the active compounds, this fraction also afforded formononetin (**9**), (+)-duartin (**10**), (±)-medicarpin (**11**), (−)-methylnissolin (**12**), (−)-melilotocarpan C (**13**), (−)-melilotocarpan D (**14**) and odoricarpan (**15**), which had no significant effects. Of the compounds isolated **2**, **3**, **6**, **7** and **15** are newly identified natural products.

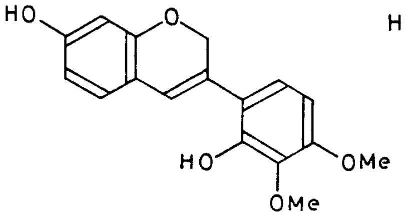
Isomucronustyrene (**2**), a colourless oil, and hydroxyobtustyrene (**3**), colourless plates mp 76-76.5°C (n-hexane/ether), showed IR, MS and ^{1}H-NMR spectra of typical cinnamylphenols 2) and were finally identified as E-1-(3-hydoxy-2,4-dimethoxybenzyl)-2-phenylethylene and E-1-(3,4-dihydroxy-2-methoxybenzyl)-2-phenylethylene by direct comparison with synthetic samples which had been prepared from corresponding phenols and trans-cinnamylalcohol according to the method of Mageswaran *et al.* 3)

(±)-Isoduartin (**6**), 4) a colourless oil, gave the spectral data very similar to those of duartin (**10**), 5) a 7,8,2',3',4'-substituted isoflavan, except for the chemical shift values of 5'-H (δ 6.44) and 6'-H (δ 6,79) signals in the ^{1}H-NMR spectrum. The MS spectrum and nuclear Overhauser effect (NOE) experiments showed that isoduartin (**6**) had 7-hydroxyl and 8,4'-dimethoxyl groups. It was suggested that the difference in the structures of duartin (**10**) and isoduartin (**6**) was the substitution at C-2' and -3'. Isoduartin (**6**) was therefore identified as 7,2'-dihydroxy-8,3',4'-trimethoxyisoflavan.

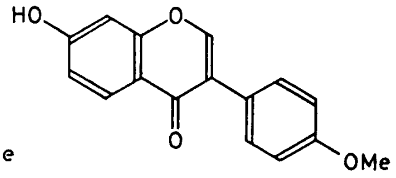
The spectral data of odoriflavene (**7**), 6) colourless prisms mp 177.5-179°C (n-hexane/AcOEt), showed that it had a 7-hydroxyisoflav-3-ene structure with an another hydroxyl and two methoxyl groups at C-2', -3' and -4'. A compound obtained by the catalytic reduction of odoriflavene (**7**) gave the same ^{1}H-NMR and MS spectra as those of (−)-isomucuonulatol which was prepared by the catalytic



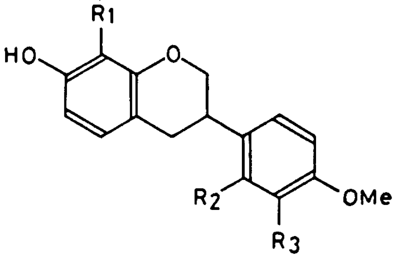
R1	R2	R3	
OH	H	OMe	obtustyrene (**1**)
OMe	OH	OMe	isomucronustyrene (**2**)
OH	OH	OMe	hydroxyobtustyrene (**3**)



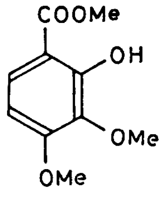
odoriflavene (**7**)



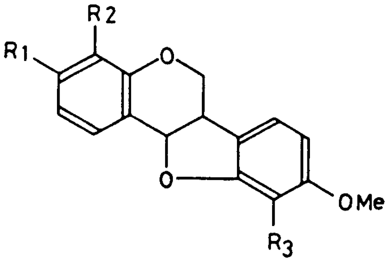
formononetin (**9**)



R1	R2	R3	
H	OMe	OH	(±)-mucronulatol (**4**)
H	OH	H	(±)-vestitol (**5**)
OMe	OH	OMe	(±)-isoduartin (**6**)
OMe	OMe	OH	(+)-duartin (**10**)



methyl 2-hydroxy-3,4-dimethoxybenzoate (**8**)



R1	R2	R3	
OH	H	H	(±)-medicarpin (**11**)
OH	H	OMe	(−)-methylnissolin (**12**)
OMe	OH	OMe	(−)-melilotocarpan C (**13**)
OMe	OH	OH	(−)-melilotocarpan D (**14**)
OMe	OMe	OH	(−)-odoricarpan (**15**)

reduction of the corresponding pterocarpan, (-)-methylnissolin (**12**). Therefore, the structure of odoriflavene (**7**) was determined to be 7,2'-dihydroxy-3',4'-dimethoxyisoflav-3-ene.

(-)-Odoricarpan (**15**), a colourless amorphous solid, gave spectral data7) very similar to those of melilotocarpan C (**13**),8) a monohydroxy-trimethoxy 3,4,9,10-substituted pterocarpan, except for chemical shift values of 1-H (δ7.34 in $CDCl_3$) and 7-H (δ6,77) signals in ^1H-NMR. Two large solvent-induced methoxy-proton shifts in the ^1H-NMR spectra measured in $CDCl_3$ and d_6-benzene9) and a positive Gibbs' test indicated the presence of methoxyl groups at C-3 and -9. These data suggested that the difference in the structures of melilotocarpan C (**13**) and odoricarpan (**15**) was the substituents at C-4 and -10. Odoricarpan (**15**) was therefore identified as 10-hydroxy-3,4,9-trimethoxy-pterocarpan. The comparison of an ORD curve with that of (-)-melilotocarpan C (**13**) shows that (-)-odoricarpan (**15**) has the 6aR, 11aR-configuration.10)

Obtustyrene (**1**), (±)-vestitol (**5**), formononetin (**9**), (±)-medicarpin (**11**), (-)-melilotocarpan C (**13**) and (-)-melilotocarpan D (**14**) were identified by direct comparison with authentic samples.11) (±)-Mucronulatol (**9**),12) (+)-duartin (**10**)5) and (-)-methylnissolin (**12**)13) were identified by comparing of their spectral data with those reported. Methyl 2-hydroxy-3,4-dimethoxybenzoate (**8**) were identified by its spectral data.14)

IC_{50} (50% inhibitory concentration) values of **1-8** against PG synthetase were 7.7, 2.8, 9.2, 63, 47, 110, 4.8 and 23μM, respectively. The cinnamylphenols (**1**)-(**3**) and odoriflavene (**7**) inhibited PG synthetase as strongly as indomethacin which is a well-known potent inhibitor with an IC_{50} value of 4.9μM under the same assay condition. Inhibition of rabbit platelet aggregation induced by ADP, arachidonic acid and collagen were assayed for **2-5**, **8-9** and **11-12**,15) which were obtained in relatively high yields among the isolated compounds. **2**, **3** and **8** were potent

Table I. Inhibitory Effects of ADP-, Arachidonic Acid-, and Collagen-Induced Platelet Aggregation

Inducer	ADP (10μM)	Arachidonic acid (128μM)	Collagen (20μg/ml)	IC 50 values against PG-ase* (μM)
Positive control	Adenosine (3.75μM)	Aspirin (11μM)	Aspirin (111μM)	
Isomucronustyrene (**2**)	>1000	52	370	2.8
Hydroxyobtustyrene (**3**)	>1000	7.7	20	9.2
(±)-Mucronulatol (**4**)	>1000	130	>1000	63
(±)-Vestitol (**5**)	1000	92	1000	47
Methyl 2-hydroxy-3,4-dimethoxybenzoate (**8**)	>1000	3.5	47	23
Formononetin (**9**)	>1000	190	>1000	>500
(±)-Medicarpin (**11**)	>1000	370	>1000	>500
(-)-Methylnissolin (**12**)	>1000	170	>1000	>500

Each figure indicates the concentration (μM) of test material which gave the same degree of inhibitory effect as positive control. * Prostaglandin synthetase.

inhibitors of PG synthetase and storongly inhibited platelet aggregation induced by arachidonic acid and collagen (Table I). Excessive platelet aggregation is concidered to cause conditions of the blood hindrance. Therefore the inhibitors of PG biosynthesis isolated from D. odorifera seem to represent effective principles against the stagnation of disorderd blood.

REFERENCES AND NOTES

1) Details of assay methods were described previously: F. Kiuchi, M. Shibuya, T. Kinoshita and U. Sankawa, Chem. Pharm. Bull., **31**, 3391 (1983).

2) 2: $C_{17}H_{18}O_3$ (M^+: m/z 270.1285, Calcd: 270.1255). IR (Cap) :3510, 1615, 1598, 1492cm^{-1}. MS m/z (rel. int. %): 270(M^+, 100), 255(16), 239(24), 167 (12), 115(35), 91(33). ^{1}H-NMR ($CDCl_3$ δ ppm): 3.48(2H, m), 3.85, 3.88(each 3H, s), 5.52(1H, s), 6.32(2H, m), 6.52(1H, d, J=8.0Hz), 6.64(1H, d, J=8.0Hz), 7.04-7.30(5H,m).

3: $C_{16}H_{16}O_3$ (M^+: m/z 256.1137, Calcd: 256.1100). IR (KBr): 3400, 1600, 1509, 1488cm^{-1} . MS m/z (rel. int. %): 256(M^+, 100), 225(15), 152(78), 115(41), 91(49). ^{1}H-NMR (d_6-benzene δ ppm): 3.31(3H, s), 3.37(2H, d, J=5.4Hz), 4.91, 4.97(each 1H, s), 6.29(2H, m), 6.54(1H, d, J=8.4Hz), 6.69(1H, d, J=8.4Hz), 6.93-7.30(5H, m).

3) S. Mageswaran, W.D. Ollis, R.J. Roberts and I.O. Sutherland, Tetrahedron Lett., **34**, 2897 (1969).

4) $C_{18}H_{20}O_6$ (M^+: m/z 332.1258, Calcd: 332.1259). MS m/z (rel. int. %): 332(M^+, 63), 180(100), 168(45), 167(79), 165(32), 164(24), 153(31), 133(45). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.90-3.05(2H, m), 3.30-3.75(1H, m), 3.85, 3.91, 3.92(each 3H, s), 4.09(1h, t, J=9.9Hz), 4.47(1H, m), 5.64, 6.00(each 1H, s), 6.44(1H, d, J=8.6Hz), 6.50(1H, d, J=8.4Hz), 6.74(1H, d, J=9.4Hz), 6.79 (1H, d, J=8.6Hz).

5) W.D. Ollis, I.O. Sutherland, H.M. Alves and O.R. Gottlieb, Phytochemistry, **17**, 1401 (1978).

6) $C_{17}H_{16}O_5$ (M^+: m/z 330.1008, Calcd: 300.0998). IR (KBr): 3245, 1610, 1495, 1460, 1430cm^{-1}. MS m/z (rel. int. %): 300(M^+, 100), 299(29), 285(27), 180(20), 143(17). ^{1}H-NMR (d_6-acetone, δ ppm): 3.80(3H, s), 3.86(3H, s), 5.02(2H, d, J=1.3Hz), 6.32(1H, d, J=2.4Hz), 6.41(1H, dd, J=2.4Hz, 7.9Hz), 6.57(1H, d, J=8.8Hz), 6.69(1H, br s), 6.95(1H, d, J=7.9Hz), 7.00(1H, d, J=8.8Hz), 8.10, 8.45(each 1H, s).

7) $C_{18}H_{18}O_6$ (M^+: m/z 330.1087, Calcd: 300.1102). $[\alpha]_D^{25}$ -136°(c=0.245, $CHCl_3$). MS m/z (rel. int. %): 330(M^+, 100), 315(67). ^{1}H-NMR ($CDCl_3$ δ ppm): 3,40-3.95(2H, m), 3.86, 3.88, 3.89(each 3H, s), 4.15-4.45(1H, m), 5.32(1H, s), 5.58(1H, d, J=6.0Hz), 6.48(1H, d, J=8.1Hz), 6.67(1H, d, J=8.7Hz), 6.77 (1H, d, J=8.1Hz), 7.34(1H, d, J=8.7Hz). ^{1}H-NMR (d_6-benzene δ ppm): 2.85-4.05(3H, m), 3.16, 3.33(each 3H, s), 3.83(3H, s), 5.11(1H, s), 5.27(1H, d, J=6.5Hz), 6.08(1H, d, J=7.7Hz), 6.33(1H, d, J=8.7Hz), 6.35(1H, d, J=8.7Hz), 6.35(1H, d, J=7.7Hz). ORD (c=0.018, dioxane) $[\alpha]^{25}$ (nm): -890°(295), -440°(286), -2110°(280), -4890°(249), -5330°(234), -7560°(222).

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R.G. Wilson, J.H. Bowie and D.H. Williams., Tetrahedron., **24**, 1407 (1968).

10) Authors are grateful to Dr. K. Takahashi (Meiji College of Pharmacy) for measurements of ORD spectra.

11) **1** was synthesized by the same method as 2. **5** was obtained by the reduction of **11**. **9** and **11** were kindly supplied by Dr. T. Kinoshita (University of Tokyo). **13** and **14** were kindly supplied by Dr. S. Fukushima (Shizuoka College of Pharmacy).

12) K. Kurosawa, W.D. Ollis, I.O. Sutherland, O.R. Gottlieb and A.B. de Oliveira, Phytochemistry, **17**, 1405 (1978).

13) D.J. Robeson and J.L. Ingham, Phytochemistry, **18**, 1715 (1979).

14) Colorless needles (n-BuOH), mp 86-87.5°C. IR (KBr): 3300, 1710, 1605, 1495, 1430, 1290, 1215cm^{-1}. ^{1}H-NMR ($CDCl_3$ δ ppm): 3.90(3H, s), 3.94(6H, s), 5.73(1H, s), 6.69(1H, d, J=9.0Hz), 7.46(1H, d, J=9.0Hz). MS m/z (rel. int. %): 212(M^+, 26), 181(43), 180(31), 151(72), 137(100).

15) Details of assay methods will be reported elsewhere.

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